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Article

Acrocarpus fraxinifolius Wight and Arn. Bark; phenolics, toxicity studies, antioxidant and anti-inflammatory activities

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The purpose of the survey was to determine acute & chronic toxicity; *in vivo* antioxidant and anti-inflammatory actions of the different extracts of A. *fraxinifolius* Wight and Arn bark; along with estimation of the phenolic, flavonoidal contents and investigation of phenolic metabolites that may attribute to the activities. LD_{50} of the total ethanol extract (TEE) was 7.1 g/kg b. wt, the radical scavenging activity of DPPH showed 60.31% inhibition, FRAP ability and $ABTS^{+}$ activity showed 55.024 and 67.217 µmol Trolox/100 g dry weight, respectively. TEE followed by ethyl acetate extract (EAE) at 100 mg/kg b.w exhibited the highest *in vivo* antioxidant activity (94.51% and 91.08% potency, respectively) compared with Vit E (100%). The TEE & EAE exhibited the highest anti-inflammatory activity (3.81±0.08 & 3.79±.0.04) respectively in comparison with indomethacin 3.83±0.01 measured as edema diameter after 4 hours of extract administration. The total phenolic and total flavonoid contents in the total ethanol extract (TEE) estimated as gallic acid and catechin equivalents were 61.06± 0.08 µg eq GA/g, 40.33± 0.20 µg CE/g extract respectively. EAE revealed five phenolic acids and eight flavonoid compounds isolated for the first time from the plant.

Keywords: *Acrocarpus fraxinifolius.* Chronic toxicity. antioxidant. Anti-inflammatory activities. Phenolics.

INTRODUCTION

A. fraxinifolius commonly known as mundane and shingle tree. It is a native spread around the world, especially in Africa and Asia (V′azquez *et al.* 1987). Pink cedar, mundane, and lazcar are common names of *A. fraxinifolius.* It is well known that the bark of any plant provides protection of the tree, structural support, and leads nutrients from the leaves to the roots, it acts as a physical and chemical barrier against microorganisms, chemically the bark has the same constituents of wood such as terpenes, polyphenols, and nitrogen containing compounds. The variation of phenolic contents of older and younger tree of the plant barks together with its *in vitro* antioxidant activities was reported (*et al.*, 2015). Considering the previous reported work for investigation of phenolics, lipoidal contents along with different biological activities of *A. fraxinifolius* (Abou Zeid *et al.*, 2011, 2012); as well as phenolic, tannins, flavonoids, *in vivo* hepatoprotective, antiproliferatives, antioxidant and antimicrobial activities of *A. fraxinifolius* leaves were studied (El-Nashar *et al.*, 2017; Walaa *et al.*, 2016). A flavone glycoside was isolated from the seeds (Bardia *et al.*, 2005) of *A. fraxinifolius* which was subjected to amino acid analysis (Bardia, Rao 2004a, b). Galactomannans from the seeds were isolated and characterized by IR spectrophotometry (Zhong, 1985). In this work we propose to yield deep insight on the phytoconstituents of A. *fraxinifolius* bark as antioxidant& anti-inflammatory active agent

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MATERIAL AND METHODS

Experimental

The structure of the compounds was identified by spectroscopic methods including: UV/VIS (Ultraviolet and Visible Absorption Spectrometer, Labomed Inc.) for measuring UV spectral data of the isolated compounds, in the range of 200–500 NM in methanol and with different diagnostic shift reagents. NMR (Nuclear Magnetic Resonance Spectrophotometer, JEOL EX, 500 MHz for determination of ^{1}H-NM Rand 125 MHz for determination of ^{13}C-NMR). Column chromatography (CC) was carried out on Flash CC (24cm long, 10cm diameter) packed with 100 g silica gel (H20 for plate). Sephadex LH-20 (Pharmazia) for purification of the isolated compounds. PC (descending) Whatman No. 1 and 3 MM papers, 15% HOAc -H_2O, BAW (n-BuOH: HOAc: H_2O 4:1:5, upper layer). Complete acid hydrolysis for *O*-glycosides was carried out & followed by Co-chromatograph with authentic samples to identify the aglycones and sugar moieties. Source of solvents used for plant extraction: SDFCL(Industrial Estate, 248 Worli Road, Mumbai-30, India).Vitamin E (dl α-tocopheryl acetate) (Pharco Pharmaceutical Co), it is available in the form of gelatinous capsules; each contains 400 mg vitamin E. As a reference drug for *in-vivo* antioxidant test. Alloxan (Sigma Co) was used for the induction of free radical *in -vivo* antioxidant. Carrageenan, Sigma Co. For induction of acute inflammation in rat, Indomethacin (Indocid), Kahira Pharm. IND. Co. A.R.E. As a standard anti-inflammatory agent.

Plant material

The leaves and bark of *A. fraxinifolius* Wight & Arn were collected in April 2008 from the Giza Zoo. The plant was identified by Mrs. Terase Labib, plant taxonomist of Orman Garden, Giza, Egypt and confirmed by the senior taxonomist, Dr. M. El-Gebaly. The plant was air-dried, powdered and kept in well-closed, dark colored containers in a cold place. A voucher specimen (M96) has been deposited by Dr. Mona Marzouk in the Herbarium of National Research Centre (CAIRC).

Preparation of total ethanol extract (TEE)

One kg of the powdered plant under investigation was extracted by 70% ethanol in a continuous extraction apparatus. After complete extraction, the solvent was evaporated under vacuum at 40 ºC (and the residue was weighed.

Preparation of successive extracts

One kg of the powdered plant was successively extracted in a continuous extraction apparatus, with the following successive solvents with increasing polarities: petroleum ether (PE), chloroform (Ch), ethyl acetate (EA) and methanol (M). After each complete extraction with one solvent, the powdered plant was dried and extracted with the next solvent. All extracts were separately evaporated to dryness and weighed.

Determination of total phenolic content (TPhC)

The total phenolic content of the total ethanol extract was determined according to the reported Folin-Ciocalteu method (Abou Zeid *et al.*, 2015). Briefly, the extract (500 µL) was transferred into a test tube and oxidized with the addition of 250 µL of Folin-Ciocalteau reagent. After 5 min, the mixture was neutralized with 1.25 mL of 20% aqueous Na_2CO_3 solution left for 40 min, the absorbance was measured at 725 nm against the solvent blank. The total phenolic content was determined by means of a calibration curve prepared with gallic, and expressed as µg of gallic acid equivalent (GAE) per mL of sample.

Determination of total flavonoid content (TFC)

The total flavonoidal content of the total ethanol extract was determined as reported (Abou Zeid *et al.*, 2015). Briefly, 250 µL of 5% $NaNO_2$ was mixed with 500 µL of extract. After 6 min, 2.5 mL of a 10% $AlCl_3$ solution was added. After 7 min, 1.25 mL of 1 M NaOH was added, and the mixture was centrifuged at 5000 g for 10 min. Absorbance of the supernatant was measured at 510 nm against the solvent blank. The total flavonoid content was expressed as µg of catechin equivalent (CE) per mL of sample.

Column chromatographic isolation and purification

Twenty five grams of the EAE were fractionated by flash CC (24 cm length, 10 cm diameter) packed with 100 g silica gel H20 for the plate. The extract was eluted with chloroform100% and increasing polarity with ethyl

acetate till 100% EA then increasing the polarity with methanol till 100% methano. A fraction of 500 mL was collected, inspected on whatman no 1 MM PC. Similar fraction were collected to give 10 major fractions (F-1 : F-10) they were then subjected to different chromatographic techniques including 3MM preparative paper chromatography and repeated Sephadex LH-20 column using eluents of different polarities this led to the isolation and purification of thirteen phenolic compounds (**1-13**). The final purification of all compounds was achieved by Sephadex LH20 column using MeOH as eluent. The isolated compounds from the bioactive EAE of *A. fraxinifolius* bark was structurally elucidated through R_f values, color reactions, chemical investigations (complete acid hydrolysis) and spectral investigations (UV, NMR and MS) (Mabry *et al.*, 1970; Markham 1982; Agrawal 1989).

Biological study

In vitro antioxidant activity

Antioxidant activity means the ability of compounds to prevent damage from reactive oxygen species (ROS) or to prevent their generation (Ku¨hnau 1976). ROS and Reactive nitrogen species (RNS) are major sources of oxidative stress in cells, damaging proteins, lipids, and DNA (Orrenius *et al,*).Therefore, prevention of oxidative stress caused by ROS and RNS has important effect for the prevention and treatment of disease (Mohammed *et al*., 2014).

Determination of DPPH radical scavenging activity

DPPH is a stable nitrogenous free radical compound, the color of which changes from violet to yellow upon reduction by either the process of hydrogen capture or electron donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers (Ebrahimzadeh *et al*., 2009b; Nabavi *et al*., 2008). Free radical scavenging capacity was determined using the stable 1,1-diphenyl-2-picryl-hydrazyl (DPPH•). The final concentration was 50 µM for DPPH• and the final reaction volume was 3.0 mL. The absorbance at 517 nm was measured against a blank of pure methanol 60 min. Percent inhibition of the DPPH free radical was calculated by the following equation: % Scavenging = **[**(A control−A sample)/ Acontrol] ×100

Where: A control is the absorbance of the control reaction

A sample is the absorbance of the test compound.

Determination of ferric reducing antioxidant power (FRAP)

The test was used to measure the total antioxidant capacity method based on the reduction of a ferric-tri pyridyltriazine complex to its ferrous colored form in the presence of antioxidants. It is regarded as accurate indicators of total antioxidant power, since total reducing power is defined as the sum of the reducing powers of the individual compounds contained in a particular sample (Tezcan *et al*., 2011).

The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL $FeCl_3 \cdot 6H_2O$ solution and then warmed at 37 ºC before using. The extract was allowed to react with 2850 µL of the FRAP solution for 30 min in the dark condition. Readings of the colored product (ferrous tripyridyltriazine complex) were then taken at 593 nm. Results are expressed in µmol Trolox/g dry matter. Additional dilution was needed if the FRAP value measured was over the linear range of the standard curve. A compound exhibiting a positive result in the FRAP assay was an electron donor and it terminated the oxidation chain reaction by reducing the oxidized intermediates into the stable form (Suganya 2007).

Determination of ABTS radical scavenging activity:

The stock solutions included, 7 m M ABTS solution and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 hours at room temperature in the dark. The solution was then diluted by mixing 1 mL $ABTS.^+$ solution with 60 mL methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using the spectrophotometer. $ABTS.^+$ Solution was freshly prepared for each assay. Plant extract (0.5 mL) was allowed to react with 3 mL of the $ABTS.^+$ solution and the absorbance were taken at 734 nm after 7 min using the spectrophotometer. Results are expressed in µmol Trolox/g dry matter

Each of the above assays was carried out in triplicate. The previous antioxidant activities were carried out according to the reported methods (Mohammed *et al*., 2015).

In vivo biological study: Experimental animals

Adult albino rats, of Sprauge Dawely Strain weighing 130-150 g and Albino mice weighing 25-30g. Animals were obtained from the animal house colony of the National Research Centre, Dokki, Egypt. They were kept under the same hygienic conditions and well-balanced diet and water. Medical Research Ethical Committee (MREC) in the National Research Centre has approved the work (15 - 101). All animals were kept at normal diet consists of vitamin mixture 1%, mineral mixture 4%, corn oil 10%, sucrose 20%, cellulose 0.2%, casein (95% pure) 10.5% and starch 54.3%. The Doses of the drugs were calculated and were administered orally by a gastric tube (Abou Zeid *et al*., 2009).

Acute toxicity test: Determination of Median Lethal Dose (LD50) Paget and Barnes 1964

Male albino mice were divided into groups, each of six animals. Preliminary experiments were undertaken to determine the minimal dose that kills all animals (LD_{100}) and the maximal dose that fails to kill any animal. Several doses at equal logarithmic intervals were chosen in-between these two doses, and each dose was injected into a group of six animals by subcutaneous injection. The mice were observed for 24 hours, symptoms of toxicity and mortality rates in each group were recorded, and LD_{50} was calculated.

Chronic toxicity test: Effect on the body and internal organ weights

Male albino rats were divided into two groups of 12 animals each. Rats of first group received 1mal saline and second groups were administered TEE of *A. fraxinifolius* bark 100 mg/kg for 8 weeks. Body weights of rats were recorded at zero, 4, 8 at the end of the experimental period (8 weeks), animals were sacrificed and their internal organs, including kidney, heart, lung, spleen liver tests and seminal vesicles were dissected off, then investigated for any morphological changes and accurately weighed. The results were illustrated in Tables I and II.

TABLE (I) – Effect of long term administration of daily oral dose (100 mg/kg B. wt.) of TEE of *A. fraxinifolius* bark on body weight in male albino rats

Group	Body weight (gm)		
	Zero time (before treatment)	4 weeks	8 weeks
Control	130.9±3.9	166.1±5.6*	187.2± 7.6*
TEE.	132.8±3.4	158.2±6.1*	186.1±8.6*

* Statistically significant from zero time at $p < 0.01$.

TABLE (II) – Relative organs weights of male rats treated with daily oral dose (100 mg/kg b.wt of TEE of *A. fraxinifolius* bark extract for 8 weeks

Organs	Relative organ weight (g/100 g B.wt).	
	Control	TEE
Kidney	0.95±0.03	0.91±0.6
Heart	0.57±0.09	0.66±0.04
Lungs	0.81±0.05	0.91±0.24
Spleen	0.43±0.09	0.46±0.09
Liver	3.90±0.03	4.08±0.30
Tests	1.50±0.02	1.8±0.07
Seminal vesicles	0.45±0.04	0.48±0.01

TEE: Total ethanol extract

Effect on biochemical parameters

Twelve male albino rats were divided into two groups of six rats. The first group kept as a control group and received 1 mL saline as a daily dose. The second received daily oral doses of 100 mg/kg b.wt. of the total ethanol extract of *A. fraxinifolius* bark for 8 weeks. Blood samples were obtained at zero time, 4 and 8 weeks from the retro orbital venous plexus of each rat, collected in clean tubes. Serum was isolated by centrifugation and divided for analysis of cholesterol Abou Zeid *et al* 2009, triglycerides, blood glucose level, creatinine, blood urea, Aspartate amino-trasferase (AST/GOT), and alanine aminotransferase ALT/GPT (Allian *et al*., 1974). Results are illustrated in Table III.

TABLE (III) – Effect of long term administration of TEE of *A. fraxinifolius* bark on cholesterol, triglycerides, glucose, creatinine, blood urea and liver enzymes (AST and ALT) serum levels in male albino rats (n=6)

Group	Time in weeks	Biochemical changes of serum levels						
Control		**Cholesterol mg/dL**	**Triglycarides mg/dL**	**Glucose mg/dL**	**Creatinine mg/dL**	**Urea mg/ dL**	**AST U/L**	**ALT U/L**
(1 mL saline)	Zero time	92.1±6.5	79.9±3.9	86.3±5.1	1.40±0.1	21.3±0.3	45.1±1.5	32.8±1.5
	4 weeks	90.2±8.6	77.2±6.1	89.6±4.7	1.42±0.2	22.1±1.7	43.2±1.3	32.4±1.3
	8 weeks	89.5±3.3	81.9±5.8	81.1±4.7	1.45±0.2	23.3±1.3	44.3±1.8	34.8±1.8
Total ext. 100 mg/kg	Zero time	88.6±2.1	84.1±1.6	89.6±2.1	1.30±0.1	21.2±1.9	44.8±1.2	33.8±1.1
	4 weeks	86.5±3.9	83.6±3.1	88.2±0.6	1.34±0.1	23.1±1.1	42.9±1.4	32.1±1.1
	8 weeks	85.8±4.5	82.9±2.2	76.3±1.2*	1.41±0.1	22.1±0.8	41.5±1.1	31.3±0.8

* Significantly different from zero time at $p < 0.05$

In vivo antioxidant activity

Forty two adult male albino rats were divided into seven groups, each of six animals, First group: received 1 mL saline and kept as a negative control. Diabetes was induced in the other groups using a single dose of intraperitoneal injection of 150 mg/kg b.wt. alloxan, followed by an overnight fast. Second group: diabetic rats that kept untreated (positive control). From third to sixth groups diabetic rats received 100 mg/kg. bwt of TEE, PEE, ChE& EAE respectively. Seventh group: diabetic rats that received 7.5 mg/kg of vitamin E as a reference drug. The rats received the extracts and the standard drug for seven days. At the end of the experiment, blood glutathione was estimated using biodiagnostic kits (Uma *et al.* 2013), the results are listed in Table IV.

TABLE (IV) – Results of *in vivo* antioxidant activity of different extract of *A. fraxinifolius* and vitamin E drug in male albino rats (n=6)

Groups	Blood glutathione (mg%)	% change from diabetic control	%Potency
Control (1 mL saline)	36.7±1.4	-----	-----
Diabetic control	21.8±0.5*	------	------
Diabetic + TEE (100 mg/kg)	35.6±1.2*	63.30	94.51
Diab. + PEE. (100 mg/kg)	32.5±1.3*	49.08	73. 28
Diab. + ChE. (100 mg/kg)	31.4±0.9*	44.04	65.76
Diab. + EAE (100 mg/kg)	35.1±1.1*	61.00	91.08
Diabetic +Vitamin E (7.5 mg/kg)	36.4±1.5*	66.97	100

* Significantly different from zero time at $p < 0.05$
TEE: Total ethanol extract, PEE: petrolium ether extract; ChE: chloroform extract; EAE: ethyl acetate extract

Determination of acute anti-inflammatory

Paw swelling, is a convenient method for assessing inflammatory responses to antigenic challenges and irritants. This effect was determined according and as reported (Abou Zeid *et al.*, 2015). This model uses carrageenan as the irritant to induce paw edema. The test materials are assessed for acute anti-inflammatory activity by examining their ability to reduce or prevent the development of carrageenan-induced paw swelling. Thirty six male albino rats weighing 130-150g were divided into six groups, each of six animals, first group received 1 mL of saline serving as control, second to fifth group received 100 mg/kg of daily dose TEE, PEE, ChE, EAE extracts respectively. The sixth group received 20 mg/kg of the reference drug indomethacin. One hour after oral administration of the extracts, all animals were given a sub plantar injection of 0.1 mL of 1% carrageenan solution in saline in the right hind paw and 0.1 mL saline in the left hind paw. The edema diameter was measured by the caliber at 1, 2, 3, 4 hours after extract administration and % edema was calculated and results are listed in Table V

$$\% \text{ Edema} = \frac{(\text{wt. of right paw} - \text{wt. of left paw})}{\text{wt. Of left paw}} \times 100$$

$$\% \text{ Edema inhibition} = \frac{(Mc - Mt) \times 100}{Mc}$$

Mc = the mean % edema in the control group
Mt = the mean % edema in the drug-treated group

TABLE V – Acute anti-inflammatory activity of the TEE extract and sucessive extracts of *A. fraxinifoliu*s bark

	Zero	1		2		3		4	
	Paw diameter (PDmm)	PD(mm) mean±SD	Oedema thickness (Oth.mm)	PD(mm) mean±SD	Oth.mm	PD(mm) mean±SD	Oth.mm	PD(mm) mean±SD	Oth.mm
Control	3.38±0.09	4.41±0.1*	1.03	4.81±0.13*	1.43	4.89±0.12*	1.51	4.96±0.08*	1.58
TEE	3.42±0.05	4.34±0.09*	0.92	4.13±0.06*	0.71	3.97±0.04	0.55	3.81±0.08*	0.39
PEE	3.45±0.6	4.31±0.12*	0.86	4.19±0.1*	0.74	4.11±0.12*	0.66	4.03±0.07*	0.58
ChE.	3.46±0.08	4.29±0.1*	0.83	4.23±0.07*	0.77	4.28±0.1*	0.72	4.24±0.07*	0.78
EAE	3.33±0.1	4.29±0.1*	0.96	4.07±0.14*	0.74	3.94±0.09*`	0.61	3.79±.0.04*	0.46
Indomet-hacin	3.56±0.08	4.26±0.09*	0.72	3.99±0.06*	0.43	3.92±0.01*	0.36	3.83±0.01*	0.27

* statistically significant different from control group at $p < 0.01$

RESULTS AND DISCUSSION

Phytochemical study

Phytochemical screening of the *A. fraxinifolius* bark revealed that the extracts prepared with polar solvents being rich in carbohydrates, flavonoides and tannins. Sterols and/or triterpenes were present in the extracts prepared with non-polar solvents.

The total phenolic and total flavonoid contents in the TEE extract were found to be 61.06±0.08 µg eq GA/g extract, 40.33±0.20 µg eq CE/g extract respectively. Chromatoghraphic isolation of major compounds from EAE (bioactive extract) of *A. fraxinifolius* led to the identification of five phenolic acids and eight flavonoidal compounds from the dried bark of the plant they are: *p*-hydroxybenzoic, *p*-coumaric acid*, protochatchuic,* sinapic, cinnamic; flavonoids: apigenin -7-O-β-

glucopyranoside, rutin, catechin, kaempferol, quercetin, chyrsin, myrcetin and luteolin. The identification was carried out through R_f values, color reactions, chemical investigations (complete acid hydrolysis) and spectral investigations (UV, NMR, MS). (Mabry *et al*., 1970; Markham 1982; Agrawal 1989) Spectral data of the known flavonoids were in good accordance with those previously published (Markham, Geiger 1994). All these compounds were isolated for the first time from the plant bark.

Biological study: Acute toxicity test

LD_{50} of the total ethanol was found to be 7.1 g/kg b. wt, this means that the plant is safe to be used in animal experiments and could be later used by humans after carrying clinical trials.

Comparison of organ weights between treated and control group of animals have conventionally been used to evaluate the toxic or adverse effects of test articles or drugs (Peters, Boyd 1966; Pfeiffer 1968).

Chronic toxicity test

Change in organ and body weight is also used as an assessment of therapeutic response to drugs (Winder 1969). The body and organ weights of experimental animals did not show any significant changes after administration of the ethanol extract for 8 weeks compared to the control groups (Table I and II). In this study the plant extract did not have any adverse effects on experimental animals that would cause them to lose appetite (El-Sanusi and El-Adam *et al*., 2007), or causing a decrease in food intake and consequently a reduction in weight with an increase in dose. Assay of biochemical parameters was performed to evaluate the liver, renal, lipid and glycemic profiles of experimental compared to the control animals, in order to give insight into pathological changes and nature of disease. In this study, assay of the liver profile parameters (AST, ALT) revealed the normal functioning of the liver after 8 weeks of administration of the aqueous extract, with reduced to normal values in experimental animals when compared to the controls.

The renal profile parameters creatinine and urea remain normal values suggest that the extract does not produce any sort of disturbance in the renal function.

The lipid profile parameters (TGY, CHOL) are indications that the extract did not exert any risk of hypercholesterolemia or atherosclerosis at low doses. The activities of AST, ALT, and the levels of urea, uric acid and creatinine showed that the function of the liver, heart and kidney are not affected by the oral supplementation of extract, Table III.

Antioxidant activity: *In vitro* antioxidants

DPPH It is evident that the radical scavenging activity of DPPH inhibition was 60.31% at concentration of 50 µg/mL for the TEE of *A. fraxinifolius* bark.

FRAP: The ferric reducing ability of TEE of *A. fraxinifolius bark* as expressed FRAP values was 55.024 µmol Trolox/100 g dry weight, thus the extract showed FRAP ability.

ABTS: The extract expressed $ABTS^+$ activity 67.217 µmol Trolox/100 g dry weight.

In vivo antioxidant

From Table IV it was concluded that 100 mg of the TEE followed by 100 mg of EAE exhibited the highest antioxidant activity as shown from the % of change of glutathione level from diabetic control (63.3 and 61.00% respectively).

Glutathione (GSH) is a natural antioxidant in the body, a lack of GSH exposes the body to the damaging effects of free radicals, thus speeding up the oxidation process. The diabetogenic process appears to be caused by immune destruction of the β cells; part of this process is apparently mediated by production of ROS. Diabetes can be produced in animals by the drugs such as alloxan which result in the production of ROS.(Oberley 1988). A simultaneous fall in blood GSH was observed following the injection of diabetogenic doses of alloxan in rabbits (Leech, Bailey 1945). The significant antioxidant activity of the EAE may be due to the presence of flavonoids (Hanasaki *et al*., 1994), such as quercetin that increases the level and the activity of GSH in mice (Molina 2003), it also has the ability to scavenge highly reactive species such as peroxynitrite and the hydroxyl radical (Boots *et al*., 2008). Catechin and other phenolic acids isolated from the plant extract were reported for their antioxidant activity (Yoshinori Kadoma, Seiichiro Fujisawa 2008).

In vivo anti-inflammatory activity

All extracts exhibited anti-inflammatory activities as indicated by inhibition of the rat paw edema weight

induced by carrageenan. The highest activity exhibited by 100 mg of the TEE & EAE 3.81±0.08 and 3.79±.0.04 respectively, in comparison with indomethacin 3.83±0.01 edema diameter as measured by the caliber after 4 hours of extract administration (Table V). Inflammation is part of the complex biological response to harmful stimuli. Pain, heat, redness, swelling, and loss of function are the primary sign of acute inflammation. The inflammation process involves activation of a complex enzyme systems mediator release, fluid extra vasations, cell migration, tissue breakdown and repair which are aimed at host defense and usually activated in most disease conditions (Turner, 1965; Vane, Botting 1995). The highest activity exhibited by TEE & EAE may be due to the presence of phenolic compounds in both extracts in addition to the nonpolar compounds in the TEE as reported (El-Rafie *et al* 2020).

CONCLUSION

Five phenolic acids and eight flavonoid compounds were isolated and identified for the first time from the bioactive EAE of *A. fraxinifolius* bark they were, phenolic acids: *p*-hydroxy benzoic, *p*-coumaric, protochatchuic, sinapic, cinnamic; flavonoids: apigenin -7-*O*-*β*-glucopyranoside, rutin, catechin, kaempferol, quercetin, chyrsin, myrcetin and luteolin. The TEE did not show any signs of acute and chronic toxicity. The encouraging results conducted with the various *in vitro* antioxidant along with *in vivo* antioxidant and anti-inflammatory activities of TEE & EAE guide us towards the possible use of the plant as safe free radical scavenger and anti-inflammatory agent.

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